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DOI: https://doi.org/10.1038/icb.2010.151

Immunology and Cell Biology (2011), 1–9

www.nature.com/icb

REVIEW

Mice with human immune system components as *in vivo* models for infections with human pathogens

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Many pathogens relevant to human disease do not infect other animal species. Therefore, animal models that reconstitute or harbor human tissues are explored as hosts for these. In this review, we will summarize recent advances to utilize mice with human immune system components, reconstituted from hematopoietic progenitor cells *in vivo*. Such mice can be used to study human pathogens that replicate in leukocytes. In addition to studying the replication of these pathogens, the reconstituted human immune system components can also be analyzed for initiating immune responses and control against these infections. Moreover, these new animal models of human infectious disease should replicate the reactivity of the human immune system to vaccine candidates and, especially, the adjuvants contained in them, more faithfully.
Immunology and Cell Biology advance online publication, 8 February 2011; doi:10.1038/icb.2010.151

Keywords: Epstein–Barr virus; HIV; dengue virus; natural killer cells; T cells; human vaccination

Mice are the preferred model species for immunological research. However, during the 65 million years of divergent evolution, mouse and man have accumulated many differences. Among all organs, most of these differences fall into olfaction, reproduction and the immune system.[1] Many of the differences in the immune system relate to innate immunity,[2] which ensures the survival of the individual during the first days after infection until the adaptive immune response can be tailored to the specific needs of the particular challenge. As mice and men encounter virtually non-overlapping groups of pathogens because of their occupation of different ecological niches, these have shaped the innate immune system of the two species quite differently. These differences become especially important, because the innate immune system initiates both rapid and adaptive immune responses, and, therefore, human immune system reactivity toward vaccines, pathogens and human disease-causing conditions in general are difficult to model in mice. In these instances, the study of human immune cells, ideally *in vivo*, would be preferable.

For this purpose investigators have transferred human immune cells into immune compromised mice since the late 1980s in order to overcome limitations of *in vitro* culture systems for human leukocytes.[3,4] However, only with the advent of the cytokine (interleukin (IL)-2, 4, 7, 9, 15 and 21) receptor common gamma chain (γ_c) knockout into mice that already lacked T and B cells because of a scid mutation or recombinase (Rag) deficiency, it has become possible to engraft significant frequencies of cells from all human immune compartments after transfer of fetal or neonatal human hematopoetic progenitor cells (HPCs) into mice.[5,6] In these mice, enhanced engraftment is generally attributed to the lack of xenoreactivity mediated by mouse natural killer (NK) cells, which seems to originate from the loss of IL-15 signaling. Accordingly, non-obese diabetic (NOD)-scid $\gamma_c^{-/-}$ or BALB/c Rag2$^{-/-}$ $\gamma_c^{-/-}$ mice are most often used for human immune compartment reconstitution from transplanted HPCs, even so some studies use parallel transplantation of human fetal liver, fetal thymus and HPCs into NOD-scid mice (bone marrow, liver and thymus (BLT)), alternatively (Figure 1).[7] Mice transplanted with human HPCs are able to reconstitute 40–60% of human CD45$^+$ mononuclear cells in peripheral blood and spleen with sizable compartments of human B cells, T cells, NK cells, monocyte/macrophages and dendritic cells (DCs) 3 month after HPC transfer (Table 1). In this review, we will summarize studies on the immunocompetence of these reconstituted human immune system components and discuss their usefulness for studying infections by human pathogens as well as vaccination approaches against these.

MODELING OF INNATE IMMUNE RESPONSES IN MICE WITH RECONSTITUTED HUMAN IMMUNE SYSTEM COMPONENTS

Immune responses start with activation of the innate immune system, which also harbors the most extensive genetic differences between mouse and man. Therefore, the reconstitution and functional capacity of innate human immune compartments in HPC-transplanted mice needs to be analyzed in detail in order to determine if such reconstituted immune cells can serve as a surrogate for human innate immune cells during studies on vaccination and infection.

Human NK cells

One of the most extensively studied cell population of the human innate immune system in mice with reconstituted human immune system components is the NK cell lineage. Initial studies mainly relied

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Received 14 September 2010; accepted 19 September 2010

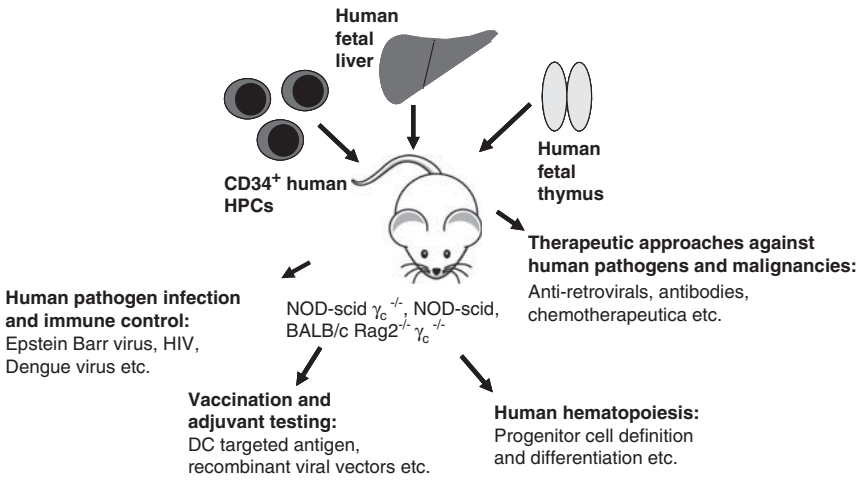

Figure 1 Main application areas for mice with reconstituted human immune system components. NOD-scid $\gamma_c^{-/-}$ (NSG or NOG), NOD-scid with human fetal liver and thymus organoid (BLT) and BALB/c Rag2$^{-/-}$ $\gamma_c^{-/-}$ mice with human immune system components, reconstituted from CD34$^+$ HPCs can be used to study human pathogen infection and immune control, vaccination with a special emphasis of adjuvant development for the human immune system, human hematopoiesis and therapeutic interventions against human pathogens and malignancies.

Table 1 Human lymphocyte compartment reconstitution after 3–6 month of human fetal HPC transfer into mice

Mouse strain	*huCD45 (%)*	*B cells*	*T cells*	*CD4$^+$ T cells*	*CD8$^+$ T cells*	*NK cells*	*Reference*
NSG (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ) or	40–60	40–60% of	30–50% of	60–75% of	25–40% of	3–5% of	Ishikawa *et al.*[5];
NOG (NODShi.Cg-Prkdc^{scid} Il2rg^{tm1Sug}/Jic)		hu-CD45$^+$	hu-CD45$^+$	hu-CD3$^+$	hu-CD3$^+$	hu-CD45$^+$	Strowig *et al.*[36]
Rag2$^{-/-}$ $\gamma_c^{-/-}$ (C.Cg-Rag2^{tm1Fwa} Il2rg^{tm1Sug}/Jic or	40–60	75–90% of	5–20% of	60–75% of	25–40% of	0.1–0.5% of	Traggiai *et al.*[6]
Stock (H2^d)-Rag2^{tm1Fwa} Il2rg^{tm1Krf}/Brn)		hu-CD45$^+$	hu-CD45$^+$	hu-CD3$^+$	hu-CD3$^+$	hu-CD45$^+$	
BLT (NOD.Cg-Prkdc^{scid} with human fetal liver and	40–60	20% of	70% of	80% of	20% of	3–5% of	Melkus *et al.*[7]
thymus organoid)		hu-CD45$^+$	hu-CD45$^+$	hu-CD3$^+$	hu-CD3$^+$	hu-CD45$^+$	

Abbreviations: HPC, hematopoetic progenitor cell; NK, natural killer.

on *in vivo* application of human cytokines and growth factors in various combinations to achieve sustained human NK cell development or an expansion of these cells to detectable levels. In the following, we will summarize these published data on development, tissue distribution, frequency, phenotype and function of human NK cells in reconstituted mice.

One of the first studies specifically addressing human NK cell development *in vivo* used adult NOD-scid mice (8 weeks of age) transplanted with CD34$^+$ HPCs from cord blood. Without treatment, no NK cells could be detected in this model. However, repetitive treatment with various cytokine/growth factor combinations lead to a transient increase in NK cells within the reconstituted mice lasting 3 weeks.[8] Of note, all cytokine combinations that led to development of human NK cells *in vivo*, contained IL-15 and flt3 ligand. Human NK cell frequencies (frequency of CD56$^+$ cells among human CD45$^+$ lymphocytes) of 4–5% could be achieved in bone marrow and spleen, and even higher percentages of $>10\%$ in peripheral blood. A CD3$^+$CD56$^+$ NKT cell population was not induced on treatment, as CD56$^+$ cells were reported to be CD3$^-$. The phenotype of the NK cells found in this study was mostly CD56^{dim}KIR$^-$ with CD16 expressed on 50–70% of all CD56$^+$ NK cells. NK cells isolated from bone marrow of these cytokine-treated mice produced IFN-γ after additional *in vitro* stimulation with IL-12 and IL-18, albeit at much lower frequencies when compared with equally stimulated NK cells from human peripheral blood. Interestingly, when *in vitro* expanded NK cells isolated from reconstituted mice were adoptively transferred into non-reconstituted NOD-scid recipients challenged with K562 tumor cells, a significant decrease in tumor burden was observed (the K562 cell line is a major histocompatibility complex-I-deficient erythroleukemic cell line). This study suggests that functional human NK cells can be reconstituted in such mice, but require additional activation by cytokines to maintain their functionality.

In contrast, when CD34$^+$ HPCs isolated from human fetal liver were transferred intrahepatically into newborn BALB/c Rag2$^{-/-}$ $\gamma_c^{-/-}$ instead of adult NOD-scid mice, another report could show multilineage reconstitution of human immune cells (absolute number of CD45$^+$ cells in the spleen around 5×10^6) and development of human NK cells at low frequencies (0.3–1.5% of human lymphocytes) in all organs analyzed.[9] These human NK cells could be divided into the two main subsets present in humans—CD56^{bright}CD16$^-$KIR$^-$ and CD56^{dim}CD16$^+$KIR$^+$ NK cells—with the former, irrespective of localization, representing the majority. This, however, is in contrast to the distribution of these two subsets in man, in which there are remarkable differences in subset ratio in different organs.[10] Of interest, NK cells almost devoid of CD56 expression could also be detected. These cells were of NK cell origin, as they expressed the NK cell-specific marker NKp46[11] as well as NKG2D, CD94 and KIRs. The human NK cell compartment in this model was functional. Interferon (IFN)-γ secretion after IL-12/-15/-18 stimulation *ex vivo* and degranulation in response to co-culture with the NK cell susceptible erythroleukemia cell line K562 in the presence of IL-15 could be detected. A direct comparison with NK cell effector functions observed in human samples was not provided. The investigators of this study furthermore report an expansion of the human NK cell compartment shortly after the repeated administration of IL-15 or IL-15/IL-15Rα hybrid molecules. This happened at the expense of a skewing toward a more

differentiated $CD56^{dim}CD16^{+}$ phenotype, converting nearly all NK cells to a $CD16^{+}$ population with accompanying acquisition of KIR expression. The duration of this increase in NK cell frequency after IL-15 treatment was not reported. Along these lines, two other studies compared NK cell reconstitution in BALB/c $Rag2^{-/-}$ $\gamma_c^{-/-}$ mice after neonatal injection with cord blood $CD34^{+}$ cells to reconstitution of this compartment on HPC transfer into NOD-scid and C57BL/6 $Rag2^{-/-}$ $\gamma_c^{-/-}$ mice.[12,13] In the latter, $CD45^{+}$ cell development did not occur at all.[12] In reconstituted BALB/c $Rag2^{-/-}$ $\gamma_c^{-/-}$ mice, the highest frequencies of $CD56^{+}$ cells were detected in lymph nodes, but the majority of these cells also expressed the T-cell marker CD3.[12] Further analysis of $CD3^{-}CD56^{+}$ NK cells showed that NK cells in the lymph node were virtually devoid of CD16 expression.[13] In the spleen, $CD3^{-}CD56^{+}$ NK cells were $CD16^{-}$ whereas in the blood most NK cells expressed CD16.[12] Administration of an adenoviral vector-encoding human IL-15 led to the expression of CD16 and KIRs on almost all splenic NK cells and to a significant increase in the frequency of $CD56^{+}$ cells in the spleen to around 2% (% of $CD45^{+}$ cells) 24 h after administration.[13] These data suggest that significant NK cell frequencies can only be achieved after HPC transfer into BALB/c $Rag2^{-/-}$ $\gamma_c^{-/-}$ mice with additional supplementation with IL-15 and that terminal differentiation of NK cells from $CD56^{bright}CD16^{-}$ NK cells can be achieved by the application of this cytokine.

NOD-scid $\gamma_c^{-/-}$ mice reconstituted with $CD34^{+}CD133^{+}$ cord blood cells injected intracardially as newborns showed a higher constitutive level of NK cells reconstitution. An expansion of NK cells in blood, spleen, bone marrow, liver and lung similar to the above mentioned was reported by Chen *et al.*[14] after hydrodynamic injection of plasmids encoding for human IL-15 and flt3 ligand. Frequencies of $CD3^{-}CD56^{+}$ NK cells of $>10\%$ (among human $CD45^{+}$ cells) were detected one week after gene delivery with a steady decline to still elevated levels over an observational period of 30 days. Nine days after hydrodynamic injection of IL-15 and flt3 ligand-encoding plasmids, the absolute NK cell number per spleen was as high as 2×10^{6}. Of note, the administration of IL-15 and flt3 ligand-encoding plasmids led to an overall increase in $CD45^{+}$ cells content in spleen (above 20×10^{6}) and bone marrow with concomitant increases in DC, monocyte/macrophage, B-cell and T-cell numbers. Apparently, the cytokine-induced NK cell expansion did not occur at the expense of a loss of the more immature $CD16^{-}KIR^{-}$ phenotype, as the majority of NK cells in bone marrow and spleen did not express CD16 or KIR molecules. However, a direct comparison with the NK cell compartment in reconstituted NSG mice without treatment was not conducted. NK cells isolated from bone marrow and spleen of reconstituted NSG mice treated with cytokine-encoding plasmids were able to specifically lyse K562 tumor cells *in vitro* and produced IFN-γ in the presence of poly(I:C) and autologous human DCs. IFN-γ levels were also increased in sera of cytokine-treated mice after *in vivo* poly(I:C) stimulation.

Moreover, hydrodynamic injection of DNA plasmids encoding granulocyte-macrophage colony-stimulating factor, IL-4 and flt3 ligand in combination, as well as macrophage colony-stimulating factor or IL-3 in combination with erythropoietin, all led to distinct increase in the frequency of DCs ($CD11c^{+}CD209^{+}$), monocytes/macrophages ($CD14^{+}$) and human erythrocytes ($CD235ab^{+}$).[14] Recently, our group reported robust and stable overall NK cell reconstitution in NOD-scid $\gamma_c^{-/-}$ mice, neonatally reconstituted with $CD34^{+}$ human fetal liver HPCs by intrahepatic injection.[15] This model allowed multi-lineage reconstitution with $CD45^{+}$ frequencies in peripheral blood of 60%. NK cell numbers in the spleen were on average 3×10^{5} cells with frequencies ranging from 1.7 to 3.4%. In peripheral blood of these mice, NK cells represented on average 5% of human lymphocytes. NK cells were detected in all organs analyzed, with the lowest frequency in the bone marrow. The analysis of the human NK cell compartment demonstrated development of all NK cell subsets present in human cord blood. Interestingly, a $CD3^{-}NKp46^{+}CD56^{-}$ NK cell subset was found that also exists in human cord blood but is present only at very low frequencies in human adult blood. This subset upregulated CD56 expression *in vivo* when transferred into autologously reconstituted NSG mice as well as *in vitro* in the presence of IL-15. In comparison with peripheral blood mononuclear cell (PBMC)-derived NK cells, a decreased functional capacity of NK cells from both mice with human immune system components and cord blood was detected. This deficit was mainly confined to the $CD56^{dim}$ and the $NKp46^{+}CD56^{-}$ NK cell subsets. Importantly, pre-activation of the human NK cell compartment from reconstituted mice and from human cord blood with IL-15 *in vitro* or poly(I:C) *in vivo* rendered these NK cells capable of secreting IFN-γ and degranulating in response to the target cell line K562. Levels of activation were similar to human adult peripheral blood NK cells. In addition, pre-activation with poly(I:C) *in vivo* led to enhanced killing of major histocompatibility complex-class I-negative tumor cells compared with parental major histocompatibility complex-class I-expressing tumor cells. These studies demonstrate that functionally competent NK cells can be reconstituted to half of the human physiological frequencies after neonatal HPC transfer into newborn NOD-scid $\gamma_c^{-/-}$ mice. Cellular frequencies can be further expanded by activation with IL-15.

In contrast, a recent report described adult NOD-scid $\gamma_c^{-/-}$ mice transplanted with mobilized $CD34^{+}$ cells from human peripheral blood donors and treated continuously with stabilized IL-7.[16] The developing NK cells in this system were reported to be functionally inert, as they lacked cytotoxic activity against K562 target cells and did not secrete IFN-γ in bulk splenocyte cultures after phorbol 12-myristate 13-acetate (PMA) and ionomycin stimulation. The phenotype of these cells was $CD56^{bright}$, mostly $CD16^{-}$ and KIR^{-}. The frequency of the NK cell compartment in this study did not exceed 5% in the organs analyzed. These data indicate that neonatal transplantation with fetal HPCs might be superior in reconstituting functional NK cell compartments in immune compromised mice.

Finally in BLT mice, which are NOD-scid mice transplanted as adults with human thymus and liver organoids under their kidney capsule followed by transfer of human fetal liver $CD34^{+}$ cells, the reconstitution of a human NK cell compartment was also independent of further addition of cytokines and reached levels of 2% of $CD45^{+}$ cells in peripheral blood 26 weeks after transplantation.[7] A detailed analysis of the NK cell phenotype and their functional capacity in this model has, however, not been reported to date.

In summary, the high reconstitution efficiency in HPC injected NOD-scid $\gamma_c^{-/-}$ mice and BLT mice, compared with other mouse models, and the presence therein of a functionally competent, but resting NK cell compartment, suggests the preferential use of these models in the analysis of the role for human NK cells in innate immune responses to infection and cancer.

Human myeloid cells

Functional data on innate immune responses mediated by the myeloid compartment of mouse models with reconstituted human immune system compartments are scarce. This is due to the fact that DCs and monocytes/macrophages are only found at low frequencies after reconstitution in immune compromised mice (3% human myeloid

cells among $CD45^+$ leukocytes, and around 1% DCs, barely detectable levels of granulocytes). One study reported the presence of $CD15^+$ neutrophils in the bone marrow at a frequency of 4% at 8–12 weeks after reconstitution of newborn NOD-scid $\gamma_c^{-/-}$ mice.[17] The same group also characterized delayed-type hypersensitivity responses in these mice after repeated challenge with trinitrobenzenesulfonate. Administration of IL-7 before immunization augmented this response, suggesting the existence of an intact innate and adaptive immune system involving macrophages, DCs and T cells. When using keyhole limpet hemocyanin as a delayed-type hypersensitivity response inducing antigen in reconstituted NOD-scid $\gamma_c^{-/-}$ mice, T cells and macrophages infiltrated the site of injection, but no swelling could be observed.[18] In a model using parallel transplantation of liver, thymus and HPC components into NOD-scid $\gamma_c^{-/-}$ mice, delayed-type hypersensitivity responses to tetanus toxoid and collagen V could be elicited with swelling and an influx of human $CD3^+$ T cells, $CD68^+$ macrophages and mouse $Ly6G^+$ leukocytes at the site of injection.[19] Furthermore, administration of the superantigen toxic-shock-syndrome-toxin-1 into classical BLT mice led to a marked increase in cytokines, an expansion of specific TCR $V\beta2^+$ T cells and the upregulation of maturation and activation markers on $CD11c^+$ DCs.[7] Moreover, our group could prime antigen-specific T- and B-cell responses in a vaccination study targeting human DCs in NOD-scid $\gamma_c^{-/-}$ mice with human immune system components.[20] These studies suggest functionality of myeloid cells and DCs during T-cell priming, delayed-type hypersensitivity and superantigen-driven immune reactions. Mice reconstituted with human immune system components might therefore be a useful tool to evaluate DC-targeted vaccine candidates.

A recently described human DC subset with functional characteristics similar to mouse $CD8\alpha^+$ DC and characterized by BDCA3 expression[21–24] can also be found in reconstituted NOD-scid $\gamma_c^{-/-}$ mice. The application of this or similar mouse models with human immune system components offers the possibility to validate the immunogenic potential of this DC subset *in vivo*. The highly translational setting of mice with human immune system components might allow testing possible roles and benefits of these cell types in new vaccination strategies.

ADAPTIVE IMMUNE RESPONSES TO INFECTIONS WITH HUMAN PATHOGENS

In addition to studying human innate immune compartments, mice with human immune system components offer the possibility to analyze infections with pathogens displaying restricted tropism for the human hematopoetic lineages, and the adaptive immune responses they evoke. Furthermore, the introduction of human leukocyte antigen (HLA) transgenes into the respective susceptible mouse strains might even allow characterizing human immune responses to pathogens with tropism for mouse somatic tissues.

Epstein–Barr virus

Epstein–Barr virus (EBV) is a γ-herpesvirus that infects >90% of the human adult population worldwide. Although EBV can infect cotton-top tamarins in experimental settings, humans are the only known natural host for EBV. Primary EBV infection can remain asymptomatic or—especially in adolescence and adulthood—can cause infectious mononucleosis. Furthermore EBV infection is associated with the development of various malignancies including Burkitt and Hodgkin lymphoma, nasopharyngeal carcinoma and lymphoproliferative diseases.[25–27] EBV has the unique ability to immortalize B cells and to transform them into lymphoblastoid cell lines. Like all human herpes viruses, EBV establishes a lifelong latent infection, mainly of B cells, within its host on primary infection.

All EBV-associated tumors express small nontranslated virally encoded RNAs, including the EBV-encoded RNAs (EBERs) and a subset of latent EBV proteins. The expression pattern of these latent proteins differs between the various malignancies. Burkitt lymphoma, for example, expresses only the nuclear antigen 1 of EBV (EBNA1) as the sole latent gene product. This expression pattern is referred to as latency I. In Hodgkin lymphoma and nasopharyngeal carcinoma, one or both of the latent membrane proteins (LMPs), LMP1 and 2, are expressed in addition (latency II). Only in tumors arising in immunocompromised patients, like human immunodeficiency virus (HIV)-infected individuals or transplant recipients, expression of all five additional EBNA proteins 2, 3A, B, C and LP can be detected (latency III). All three types of EBV latencies can also be found in healthy EBV carriers and this EBV protein expression pattern seems to depend on the differentiation stage of the infected B cells.[28,29]

Immune control of EBV is mainly mediated by T cells.[30,31] As this cellular subset of human lymphocytes poorly reconstitutes and survives in the xenogeneic mouse environment, experimental studies on EBV immune control in mice have long been limited. As early as 1990, it was shown that transfer of PBMC from healthy EBV carriers to scid mice leads to the development of tumors that in many aspects resemble EBV-driven lymphoproliferative disease (LPD).[32] However, the poor survival of human T cells within the transferred PBMCs did not allow any assessment of T-cell-mediated immune control. Nevertheless, autologous *ex vivo* expanded EBV-specific cytotoxic T lymphocytes (CTLs) did home to sites of tumor formation in this model and specifically killed infected tumor cells.[33] The first mouse strain allowing direct EBV infection in mice after reconstitution with human immune cells was the NOD-scid mouse.[34] On injection of HPCs, human B cells develop in these animals and can be infected with EBV by injection of virus containing culture supernatants. At 4–6 weeks after infection, mice showed disseminated LPD and high titers of viral DNA could be detected in peripheral blood. Like most EBV-driven tumors found to date in mice with human immune system components, these tumors expressed EBERs, LMP1 and EBNA2, thereby indicating latency III. Residual mouse NK cell activity in NOD-scid mice, however, limits the development of human T cells and thereby hampers attempts to analyze T-cell-mediated immune control.

As discussed above, the additional knockout of the common γ-chain in NOD-scid as well as in BALB/c $Rag2^{-/-}$ mice minimizes residual murine NK cell activity and thus allows robust T-cell development after reconstitution with human HSC. In a seminal publication Traggiai *et al.*[6] could show stable human T-cell homeostasis in BALB/c $Rag2^{-/-}$ $\gamma_c^{-/-}$ mice reconstituted with human HPCs. These T cells expand upon EBV infection. As similar to primary EBV infection in humans, the vast majority of expanding T cells are $CD8^+$ T cells, this induces an inversion of the $CD4^+$ T cells/$CD8^+$ T cells ratio in the spleen. T cells isolated from infected mice produce IFN-γ when stimulated with autologous lymphoblastoid cell lines, thereby proving *in vivo* T-cell priming. Similar findings were also reported using BLT and NOD-scid $\gamma_c^{-/-}$ mice.[7,35] The latter mouse strain has been used by our group and others to further characterize EBV infection and immune control in reconstituted mice. EBV infection in these mice shows a dose-dependent effect. Low-dose infection (10^4 Raji infectious units) leads to T-cell reactivities against lymphoblastoid cell lines and asymptomatic EBV persistence. In contrast, intermediate- and high-dose EBV infection (10^5 and 10^6 RIU (Raji infectious units), respectively) results in a stronger expansion of EBV-specific T cells and especially high-dose infection even

results in EBV-driven tumor formation. Expanded T cells furthermore show direct cytotoxicity against autologous lymphoblastoid cell line and their EBV-epitope specificity could be identified by peptide stimulation. Among expanded T cells, high frequencies of $CD45RO^+$ and $HLA\text{-}DR^+$ cells can be detected, indicating an activated, memory T-cell-like phenotype.[35,36] Research from our group demonstrated that the observed priming of EBV-specific T cells is of protective value.[36] Depletion of human T cells before EBV infection resulted in the development of disseminated LPD and higher viral DNA loads in the spleen of infected animals at early time points after infection. Of note, $CD4^+$ T-cell depletion alone as well as sole $CD8^+$ T-cell depletion lead to a significant increase in viral DNA load. Yajima *et al.*[37] who reported a more rapid onset of LPD and a shorter lifespan in reconstituted EBV-infected mice after T-cell depletion, confirmed the protective value of T cells in this system.

However, human T cells in reconstituted NOD-scid $\gamma_c^{-/-}$ and $Rag2^{-/-}$ $\gamma_c^{-/-}$ mice are selected under suboptimal conditions on mouse thymic-epithelial cells and human bone marrow-derived cells in the murine thymus. This unphysiological selection process, which includes selection on human major histocompatibility complex as well as on mouse H2 molecules, seems to result in different affinities and specificities than T cells selection in an all-human setting. This might explain why the detected T-cell specificities were mostly directed against subdominant EBV epitopes. Such limitations can be overcome by the introduction of human HLA transgenes into the mouse genome. After insertion of a human HLA-A2 heavy chain transgene into NOD-scid $\gamma_c^{-/-}$ mice (NSG-A2 mice), immuno-dominant peptide specificities against EBV lytic and latent antigens can be detected at relatively high frequencies.[36,38]

In summary, mice reconstituted with human immune system components model many aspects of EBV infection and immune control that can also be found in humans. EBV infection of such mice leads to T-cell expansion with an inversion of the $CD4^+/CD8^+$ T cell ratio and a predominance of activated memory-like T cells. It primes protective antiviral T-cell responses in a dose-dependent manner and primed T cells recognize EBV-derived epitopes that can also be found in human EBV carriers. At higher viral doses, EBV can cause LPD formation and by their expression of latent EBV proteins, these LPDs resemble tumors that are normally found in immunocompromised patients.

For the future, mouse models with reconstituted human immune system components offer many interesting possibilities for research on EBV biology and immune control. Additional latency patterns associated with different EBV-associated malignancies might be accessible in these mice under certain infection conditions.[39] If the exact requirements for these could be further defined, immune control of EBV malignancies associated with these 'non-type III' latencies including Hodgkin disease and Burkitt's lmphoma could be studied in greater detail.

Mice with reconstituted human immune system components could furthermore serve as a model for preclinical drug testing. Until now, specific drug treatment of EBV-associated diseases relies mainly on inhibitors of viral DNA polymerases like Aciclovir. DNA polymerase inhibitors are effectively reducing viral DNA synthesis during lytic replication of EBV, but they fail to effectively target EBV in latently infected cells. To circumvent this problem, one arm of current research focuses on the development of small molecules targeting latent EBV proteins like EBNA1 and inhibiting their function.[40] Mice with human immune system components can help to test safety and efficacy of these and other new drugs before they enter tests in primates or even humans.

In addition, such models might allow for the first time the assessment of the *in vivo* biology and the immune control of genetically modified EBV strains. Various modified EBV strains derived from artificial bacterial chromosomes have been developed over the last decade. So far the effect of targeted mutation or deletion of specific EBV genes could only be addressed *in vitro*. How would, for example, modifications of the viral lytic cycle by deletion of the main lytic transactivator BZLF-1[41] affect virus persistence and T-cell priming? Or how would the loss of immuodominant proteins like EBNA3B[42] affect tumor development and immune surveillance *in vivo*? These and other questions could not be addressed so far. Mice with human immune system components will help to find answers to some of those questions and will hopefully broaden our understanding of the complex interaction EBV has with the human body.

Dengue virus

The four serotypes of dengue virus (DENV) belong to the family of Flaviviridae, which are small enveloped RNA viruses. They are transmitted by mosquito bites and global incidence has increased drastically over the past decades, especially in Asia. Infection can lead to a flu-like disease called dengue fever. Symptoms range from mild-to-severe and include fever, rash, headache, nausea and vomiting as well as muscle and joint pain. The cellular basis and contribution of the host's immune system to disease are not understood in detail. In some cases and especially after secondary infection with another serotype, patients develop dengue hemorrhagic fever with thrombocytopenia and plasma leakage, which can be fatal. To date, there is no specific antiviral treatment or licensed vaccine available partly because of the lack of an appropriate animal model. Non-human primates support productive infection, but they do not show symptoms of dengue fever or dengue hemorrhagic fever. Several mouse models have been used to study aspects of DENV infection in the past, however, the relevance of the results for the human setting is limited. Infectious doses were un-physiologically high or the mice were not fully immunocompetent. Furthermore, mouse-brain-adapted virus strains were used or virus was injected into the central nervous system directly. To overcome some of these limitations, Bente *et al.*[43] infected NOD-scid mice reconstituted with $CD34^+$ cord blood cells with DENV serotype 2 subcutaneously to mimic mosquito bites. Mice developed symptoms associated with dengue fever like an increase in body temperature, rash and thrombocytopenia. Mota and Rico-Hesse[44] used reconstituted NOD-scid $\gamma_c^{-/-}$ mice to compare the severity of these symptoms induced by different strains of DENV serotype 2 after a low dose, subcutaneous injection. In accordance with experiments in human target cells, a Southeast Asian strain was the most virulent one in this mouse model. It was also the only strain that induced the production of DENV-specific antibodies. Also reconstituted $Rag2^{-/-}$ $\gamma_c^{-/-}$ mice support DENV infection. Similarly, they develop fever, but unlike reconstituted NOD-scid $\gamma_c^{-/-}$ mice, they do not display signs of rash or hemorrhage after combined intraperitoneal and subcutaneous DENV injection. They did, however, generate DENV-specific immunoglobulin (Ig)M and IgG antibodies, which in part had neutralizing capacity.[45] It is also possible to generate a DENV-specific T-cell response in reconstituted mice. When Jaiswal *et al.*[46] infected HLA-A2 transgenic NOD-scid $\gamma_c^{-/-}$ mice reconstituted with HLA-A2-positive HPCs, they detected T cells that secreted IFN-γ, IL-2 or tumor necrosis factor-α after restimulation with a DENV peptide pool.

In conclusion, there are differences in symptoms and the time period of the immune response to DENV in the reports discussed above, which are most likely because of the use of different mouse

models of human immune components reconstitution. Nonetheless, reconstituted mice seem to emerge as a good model to study the pathogenesis of DENV and should help discover therapeutic as well as preventive strategies.

HIV

Despite all efforts, infection with HIV and the resulting immunodeficiency are still a major threat to global health. In 2008, 2.7 million people were newly infected with HIV, with a total of 33.4 million people being HIV-positive worldwide (World Health Organization). The most common form of transmission is by sexual intercourse. In the acute phase of infection, HIV levels in plasma peak. Subsequently, HIV levels drop, most likely because of a $CD8^+$ T-cell response that eliminates infected cells. The major target cells are $CD4^+$ T cells, but also monocytes, macrophages and DCs can be infected. In the latent phase of infection, the human immune system controls viral infection, resulting in low viral levels in plasma. Over time, $CD4^+$ T-cell levels decrease causing the immune system to lose functionality. This gives rise to opportunistic infections and cancer development that eventually will cause death. Combined antiretroviral therapy delays disease progression, but there is no cure or vaccine available to date. Major problems pose the integration of the viral DNA into the host DNA for long-term viral persistence and the high mutation rate of the virus facilitating immune evasion. Furthermore, we lack a suitable animal model to study HIV infection and to test new antiviral therapeutics and vaccines. Experiments in chimpanzees, macaques, cats, rats and mice have allowed insights into several aspects of HIV biology.[47] However, HIV pathogenesis in these models as well as the resulting immune response differs from human infection. To overcome these problems, several mouse models with reconstituted human immune system components have been tested to date. Early experiments were carried out in scid mice with a human fetal thymus and liver transplant (SCID-hu thy/liv mice). HIV isolates could infect T cells and thymocytes in the engrafted human thymus of these mice and infection lead to T-cell depletion.[48–51] Therefore, this model allows *in vivo* drug testing and the study of viral cytopathogenicity. The major limitation is the lack of a complete immune system with circulating lymphocytes and human antigen-presenting cells. In contrast, more cellular compartments can engraft in scid mice, when transplanted with human peripheral blood mononuclear cells (SCID-hu-PBL). Nevertheless, this model mainly allows the analysis of the acute phase of infection and the evaluation of antiretroviral therapy, as also here $CD4^+$ T-cell depletion is rapid and T cells are not replenished.[52,53]

In the past few years, several mouse models were tested for HIV infection that have the capacity to renew their human T-cell compartment after reconstitution of human immune system components from HPCs, namely $Rag2^{-/-}$ $\gamma_c^{-/-}$, NOD-scid $\gamma_c^{-/-}$ and BLT mice. All models support viral infection with viremia in the plasma, show dissemination of the virus to various organs and exhibit $CD4^+$ T-cell depletion.[54–61] Most importantly, all these reconstituted mice mount at least weak immune responses to HIV. Several groups detected a humoral immune response, however, at low frequencies. For example, Baenziger *et al.*[54] reported one IgG producing mouse after HIV infection in reconstituted $Rag2^{-/-}$ $\gamma_c^{-/-}$ mice. In another study, 3 out of 14 reconstituted NOD-scid $\gamma_c^{-/-}$ mice produced antibodies that were specific for env gp120 or gag p24.[57] In the same mouse background, Sato *et al.*[62] found IgG in seven out of seven infected mice (data shown for two), which were in part specific for gp41. One reason for a higher IgG frequency in this study might be the long period of time between infection and analysis. Also the majority of infected BLT mice generated HIV-specific antibodies with epitopes including gag, nef, pol and env after prolonged time periods of infection.[63] In addition to a late time point for analysis, having a human thymus in BLT mice might allow the generation of more potent T helper cells and thereby more frequent antibody responses.

With respect to the T-cell compartment and cellular immune responses to HIV in reconstituted mice, the characteristic feature in all reported studies is the loss of $CD4^+$ T cells. For example, infection with CXCR4-tropic HIV induced fast depletion of both naïve and memory T cells. In contrast, $CD45RO^+$ effector memory T cells were preferentially and gradually lost in CCR5-tropic HIV-infected mice.[64] Furthermore, $CD25^+$, $FOXP3^+$-positive regulatory T cells are also preferentially depleted by HIV infection in $Rag2^{-/-}$ $\gamma_c^{-/-}$ mice.[65]

On the other hand, HIV infection induces proliferation of $CD8^+$ T cells. At least in reconstituted NOD-scid $\gamma_c^{-/-}$ mice, this could mainly be accounted for by an increase in $CD45RA^-CD8^+$ memory T cells.[62] In BLT mice, Sun *et al.*[60] reported activated, $CCR5^+$, as well as granzyme and perforin-positive $CD8^+$ T cells in lymph nodes after HIV infection. Accordingly, Brainard *et al.*[63] found a significant increase in activated CD69 and HLA-DR-positive $CD8^+$ T cells as well as perforin-positive $CD8^+$ T cells in HIV-infected BLT mice in comparison with uninfected mice. In addition, they detected HIV-specific T-cell responses 9 weeks after infection. $CD4^+$ as well as $CD8^+$ T cells produced IFN-γ after *ex vivo* peptide restimulation.[63] Interestingly, gag and nef were the most frequently recognized T-cell antigens in these mice as is the case in human HIV-infected patients. Despite this robust immune response, also the BLT mice of this study did not show decreased viral load after T-cell priming. One possible explanation might be emerging T-cell exhaustion. As in chronic human infection, HIV lead to an increase in frequency and expression levels of PD-1 on $CD4^+$ and $CD8^+$ T cells in BLT mice. Furthermore, there was a positive correlation between the percentage of PD-1-positive cells and plasma viremia,[63] which supports this hypothesis.

In line with the findings in BLT mice, reconstituted NOD-scid $\gamma_c^{-/-}$ mice mount a strong HIV-specific cellular immune response. After restimulation with a gag-derived peptide pool, $CD4^+$ and $CD8^+$ T cells of most infected mice produced IFN-γ and a subset of these cells was also producing IL-2.[66] The importance of the $CD8^+$ T-cell response was underscored by depletion experiments. Although administration of CD8-depleting antibody several weeks after infection only had a mild effect on viral load, viremia was significantly increased when $CD8^+$ T cells were depleted 2 weeks after infection.[66]

In all studies mentioned above, mice were infected with CXCR4- and/or CCR5-tropic HIV by intraperitoneal injection. For a more physiological way of exposure, Sun *et al.*[60] infected BLT mice intrarectally after abrasion of the rectal epithelium. Like intraperitoneal injection, this lead to viral spread throughout the body and $CD4^+$ T-cell depletion. The same can be achieved by intravaginal infection.[67] Of note, pre-exposure prophylaxis with antiretroviral drugs prevented vaginal HIV transmission. Rectal and vaginal transmission has also been shown for $Rag2^{-/-}\gamma_c^{-/-}$ mice,[55] but could not be reproduced by another group possibly because of very low overall levels of reconstitution in this particular mouse background.[68]

Taken together, the newer mouse models with reconstituted human immune system components recapitulate various aspects of HIV infection in humans and even allow the study of long-term chronic HIV infection.[69] Better immune control might be achieved after improved reconstitution in transgenically modified mice expressing human HLA molecules or cytokines. Of course, one has to keep in mind that various cofactors have a role in acquired immune deficiency syndrome progression. Only few studies tried to address an impaired

integrity of the intestinal barrier as seen in patients.[70] Additionally, coinfection with other pathogens will need to be studied more intensively.[71–73] Nonetheless, mice with human immune system components are useful tools to test antiretroviral therapeutics and possibly vaccines.[47,67,74–77] However, it will be important to test compounds in mice and patients side by side to determine whether results in mice with human immune system components can be extrapolated to the human setting.

VACCINATION IN MICE WITH HUMAN IMMUNE SYSTEM COMPONENTS

Mice with reconstituted human immune components can serve as preclinical surrogate models to investigate the pathogenesis of viral infections and other human diseases. These models are new tools in the development of effective and affordable vaccines and therapeutics.

Vaccination against EBV-associated diseases

Although over the past years different therapeutic vaccination approaches against EBV-positive malignancies were able to lead to an expansion of T cells specific for viral antigens, no study so far has proven to be clinically effective against Hodgkin's lymphoma and nasopharyngeal carcinoma.[78,79] In a nasopharyngeal carcinoma trial using *ex vivo* generated DCs pulsed with peptides derived from EBV proteins, expansion of EBV-specific $CD8^+$ cytotoxic T cells could be detected. Clinically, however, no remission or slowing of tumor growth could be observed.[78] These data indicate that it is crucial to test whether the primary T-cell responses elicited *in vitro* will confer protection against virally transformed cells and tumors *in vivo*. As EBV-specific T-cell responses limit viremia and tumor formation during infection in reconstituted NOD-scid $\gamma_c^{-/-}$ mice,[36,37] this particular mouse model seems suitable to test vaccine candidates that aim to induce protective T-cell responses. Along these lines, we tested vaccination with an EBNA1 fusion antibody targeting the endocytic DEC-205 receptor on human DCs together with the application of a TLR3/mda5 agonist as adjuvant in such reconstituted mice. In response, they elicited EBNA1-specifc T-cell response as well as anti-EBNA1 antibodies.[20] However, the observed levels of T-cell reactivity in most vaccinated mice were 10-fold lower than the levels of reactivity observed during intermediate-dose EBV infection.[36] Therefore, these encouraging results will need further improvement before vaccinated mice can be challenged with high-dose EBV infections. Such improvements might include selection of more efficient adjuvant formulations to boost DC maturation for more efficient T-cell priming after antigen has been targeted to them.

Vaccination against HIV

Lots of attempts to generate a vaccine against HIV-1 have failed despite more than 20 years of effort. The virus envelope has evolved to evade neutralizing antibodies and continuous mutations of the virus are enabling it to evade anti-HIV T-cell responses.[80] Two highly publicized HIV vaccine trials were prematurely terminated because of a high frequency of sero-conversions among vaccine recipients, indicating non-effectiveness of the vaccine.[81] With these disappointing results, there has been increased interest in rodent models with human immune system components for HIV infection in order to test preclinical vaccine candidates. Earlier studies using SCID-hu-PBL mouse models showed that high doses of the neutralizing human monoclonal antibody IgG1b12 can block viral entry and thereby can protect the host from developing high plasma viremia.[82,83] However, the transferred PBMCs in this mouse model did not sustain T-cell reconstitution and, therefore, HIV-1 infection. Over time, such mice furthermore suffer from a severe (xeno-) graft versus host disease, because of high xenoreacitivy of the transplanted human PBMCs against the murine host's cells. Rag2$^{-/-}$ $\gamma_c^{-/-}$, NOD-scid $\gamma_c^{-/-}$ and BLT mice, on the contrary, support long-lasting and robust *in situ* development of human hematopoietic cells, relevant to HIV infection.[54,57,59] However, the immune responses in these mice are still suboptimal with both humoral and adaptive HIV-specific immune responses only developing after several months of HIV infection. Nevertheless, the observation that $CD8^+$ T-cell response at least partially control viremia in reconstituted NOD-scid $\gamma_c^{-/-}$ mice,[66] might offer the opportunity to test vaccine candidates in at least this particular mouse background in the future.

Vaccination against other pathogens

Apart from testing potential vaccine candidates against EBV and HIV, mice reconstituted with human immune system components have also been employed to test reactivities elicited by established vaccines or new vaccine candidates against other pathogens. Mice with human immune system components were used to test vaccines against severe acute respiratory syndrome-associated corona virus. Okada *et al.*[84,85] transfused PBMCs from healthy human volunteers into NOD-scid $\gamma_c^{-/-}$ mice and immunized these mice with complementary DNA constructs encoding the structural antigens of the virus. After three immunizations, the candidate vaccine could induce a human neutralizing antibody response and a modest cytotoxic T-cell response specific to the virus. However, the xenorecognition of the mouse host by the transferred PBMCs could augment adjuvant activity of the vaccine. This activation would not be observed during vaccination of humans.

Rapid allo- and xenorecognition is mostly mediated by human memory T cells. To circumvent this reactivities, cord blood mononuclear cells, which almost exclusively consist of naïve T cells, have been used to engraft NOD-scid mice in other studies. This model was for example used to test an adenoviral cancer vaccine to human carcino–embryonic antigen. Camacho *et al.*[86] demonstrated that engrafted human lymphocytes responded to the cancer vaccine by producing IFN-γ when the restimulated *in vitro* with carcino–embryonic antigen peptides. However, the protective value of the induced T-cell responses was not explored and even naïve, but fully educated human T cells might raise xenoreactive immune responses toward their mouse host over time, thereby at least in part contributing to the observed T cells activation. In newborn mice transplanted with human HPCs, developing human lymphocytes are tolerized against mouse tissue because of their selection in the mouse thymus. Therefore, these models might be more favorable for vaccine studies.

Traggiai *et al.*[6] vaccinated reconstituted BALB/c Rag2$^{-/-}$ $\gamma_c^{-/-}$ mice with tetanus toxoid and this immunization induced measurable anti-tetanus toxoid IgG antibodies. However, the detected antibody levels were significantly lower than those achieved in human adults. Furthermore, a recent study showed that NOD-scid $\beta2m^{-/-}$ mice engrafted with adult human $CD34^+$ HPCs and further reconstituted with human T cells, can mount specific immune responses against influenza virus vaccines.[87] Influenza matrix protein 1-specific $CD8^+$ T cells were expanded in mice vaccinated with inactivated trivalent influenza virus vaccine. The expansion of these antigen-specific $CD8^+$ T cells required reconstitution of the human myeloid compartment. Nevertheless, the protective value of this vaccine response against influenza virus infection was not investigated in this study.

Taken together, mice with reconstituted human immune system components are now able to model certain aspects of infections with human pathogens and the specific immune control of these.

However, immunity following vaccinations remains suboptimal and the potency of novel vaccine candidates, especially to trigger cell-mediated immune responses needs to be further improved, before the protective value of these vaccine candidates can be evaluated by subsequent infection with human pathogens.

CONCLUSIONS

With the advent of new mouse strains showing improved tolerance toward human immune system component reconstitution *in vivo*, most human immune compartments can now be reconstituted for prolonged periods of time *in vivo*. These mouse models can serve as tools to study infection and to some extent immune control of human pathogens *in vivo*. Although innate immune responses and T-cell responses can probably be modeled fairly well, the lack of steady-state germinal center formation and the associated defect in isotype switching and affinity maturation of humoral human immune responses, will probably limit the usefulness of these new models against pathogens that require antibody-mediated neutralization for immune control. However, these models might be rather suitable to study pathogens like EBV that are primarily controlled by T-cell-mediated immunity. Furthermore, vaccine development that aims to primarily evaluate antigen plus adjuvant candidates for improved T-cell immunity might be advanced in these mice. As pathogen-associated molecular pattern recognition differs between mouse and man and strongly affects adjuvant recognition during vaccination, vaccination studies might especially benefit from these new models.

ACKNOWLEDGEMENTS

SM and CSL are recipients of postdoctoral fellowships of the German Research Society (DFG) and Croucher Foundation, respectively. Work in our laboratory is in part supported by the National Cancer Institute (R01CA108609 and R01CA101741), the Foundation for the National Institutes of Health (Grand Challenges in Global Health), the Swiss National Science Foundation (310030_126995) and the Sassella Foundation to CM.

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